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Folic Acid and Its Receptors

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Overview of Folic Acid

Folic acid, also known as folate or vitamin B9, is essential for various functions in the human body and life as we know it. Folate is the compound that occurs naturally in food, and folic acid is the synthetic form of this vitamin (1). Chemically speaking, folic acid has the hydrogen (H+) attached to the compound whereas folate is the conjugate, having lost the hydrogen (H+) (1). In the discussion below, folic acid and folate will be used interchangeably. The human body requires about 400 micrograms of folic acid daily, but cannot create folic acid on its own; instead the human diet must take in folate on a daily basis (2). Foods high in folate include leafy greens, egg yolks, beans, lentils, and sunflower seeds (3). Folic acid is crucial in many bodily processes, including DNA synthesis, DNA repair, DNA methylation, and the production of red blood cells. A lack of folic acid, called folate deficiency anemia, has been linked to spinal and neurological birth defects, infertility in both genders, depression, and tumorogenesis (2). It can be caused by various diseases of the small intestines, which prevent folic acid absorption, or by a lack of folate intake. Either way, about 75% of American take in less than the recommended 400 micrograms of folate per day (2). Because folic acid is easily broken down and lost through the preparation of food, (cooking, steaming, frying, and baking) fortified folic acid is added to many foods we eat, most commonly to wheat. Pteroylmonoglutamate is the synthetic form of folic acid, which is added to fortified foods (4).

Being in its oxidized state, it contains only one conjugated glutamate residue, instead of the two glutamate residues folate normally has (4). Fortified folic acid must therefore enter the cells via a different carrier system then naturally occurring folate uses (5). Folic acid has a higher bioavailability than folate and is more rapidly absorbed across the intestine (4).

Structure

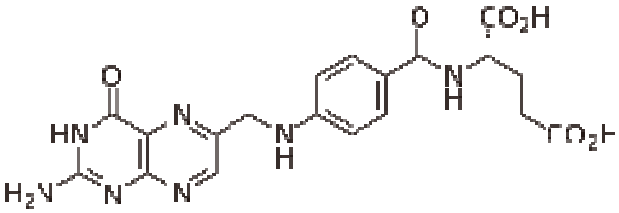

Figure 1(5).

The chemical makeup of folate is C19H19N7O6 and at room temperature is a yellow-orange crystaline powder. The chemical structure includes the aromatic pteridine ring bonded to para-aminobenzoic acid with terminal glutamate structures (5). While the aromatic ring structures are non-polar, folic acid is water soluble due to the glutamate residues on the terminal and the various nitrogen elements throughout the compound. It has a pKa of 4.65, meaning that it is a weak acid in water (6). Folic acid is not biologically active until converted to dihydrofolic acid in the liver by dihydrofolate reductase by means of the folic acid cycle (5,6).

Folic Acid Cycle

The folate cycle allows demethylated methionine to become metholated by folic acid, with help from various cofactors and enzymes. Methionine is an essential amino acid that is required for proper protein assembly and function (7). To start the folic acid cycle, demethylated

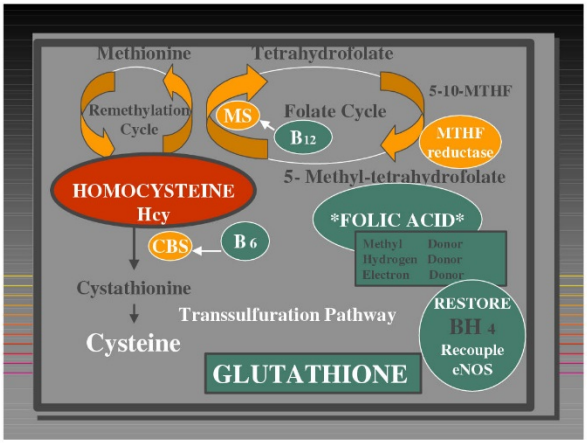

Figure 2 (6).

methionine enters for remethylation with the help of folic acid. To allow this to happen, the enzyme methionine synthase, and cobalamin (vitamin B12) are also required. Folic acid acts as a methyl-donor and as electron donor to restore the required BH4 cofactor and to recouple the eNOS enzyme that is required for eNO production from L-arginine (8). Hcy then enters the transsulfuration pathway with the assistance from cystathionine beta synthase (CBS) enzyme and the required cofactor B6 to form cystathionine and cysteine. Excess cysteine waste from the cycle is excreted through the urine after being used to make glutathione. Folic acid and cofactors B6 and B12 are necessary for improving hyperhomocysteinemia and recoupling the BH4 cofactor to the eNOS enzyme for the production of eNO to allow the cycle to happen again (9,10).

Functions of Folate

Folic acid is necessary for many vital reactions in cells to allow life to occur. Tetrafhydroformal folic acid (the biologically active form) allows for one carbon structures to be added to other molecules to form essential nucleotides and amino acids in the cell (11). Once folic acid allows amino acids to form, protein metabolism can commence and allow other biological functions to happen. Besides amino acid formation and protein metabolism, folate also allows for the reduction of blood homocysteine levels by methylating the thiol group, preventing homocysteine from forming (12, 13). Allowing for the formation of red blood cells, a lack of folic acid can cause folate deficiency anemia. Folate is needed for the formation of heme, the iron-containing segment of hemoglobin in erythrocytes. A deficiency impairs the maturation of young red blood cells, which results in anemia (14). As a requirement for cell growth and division, a lack of folic acid will cause defects in cell growth, especially in infants

whose mothers lacked folic acid (15). High amounts of folic acid taken during pregnancy will prevents neural tube defects and anencephaly (16).

Folic Acid in Bacteria

Unlike humans, most forms of bacteria, as well as plants and fungi, have a very unique folic acid property: the ability to create folate on their own. Folate cannot cross cell walls by diffusion or active transport, into or out of the cell. Cell walls are made of peptidoglycan, cross-linked sugars and amino acids that does not allow for folic acid, or other polar molecules, to pass through (17). Since plant cells, fungi cells, and bacteria all have cell walls, these cells make folate *de novo* (18). These organisms start with GTP and chorismate to create folate, but higher organisms (mostly animals) lack the necessary enzymes to create this vitamin, and so they must aquire it through diet (19-22). Bacteria synthesize folic acid from p-aminobenzoic acid, using the *orf17* gene (17), and the *fmt* gene (23), among many others that have yet to be determined (23). First isolated in E. coli, a full knockout of the *orf17* gene causes bacteria to create at least 10 times less folate than usual (27), which shows that it is essential to folate synthesis in bacteria. In genetic sequencing, it was found that the same folQ gene allowed for folate synthesis in both plants and

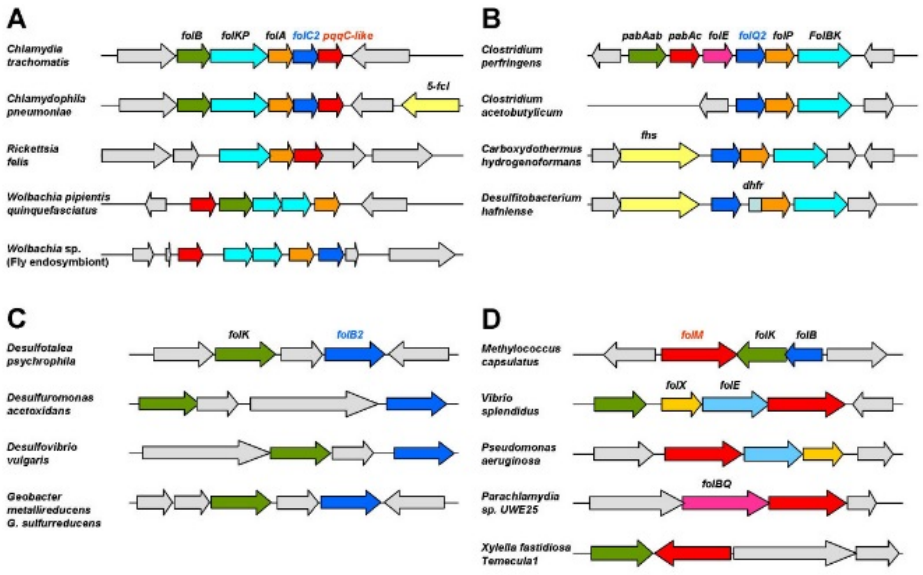

Figure 3 (23). This figure shows various bacteris and the known folate-related genes required for *de novo* folate production. Genes which are orthologous have matching colors. The grey arrows are for non-folate related genes.

bacteria (28). Figure 3 shows various genes that have been found to be required in *de novo* synthesis of folate (23). *Para*-4-aminobenzoic acid, or PABA, is the essential vitamin required by most bacteria, including *E. coli,* to create folate (28). These bacteria synthesize PABA from chorismate using PABA synthase (29), which is also the precursor to aromatic amino acids (30).

There are a few bacteria strains that cannot synthesize folate on their own and must take it in from an external source. These bacteria that lack the ability to make it *de* novo since they lack the protein and associated gene required, such as HPPK/FolK and DHPS/FolP. *Mycoplasma* and *Treponema, Lactobacillus casei* and *L. salivarius,* along with other organisms that live in folate-rich environments, are examples of such bacteria strains (23). There are also certain plants that also live in folate-rich environments and the chloroplasts and vacuoles of said plant cells must take up folates from the cytoplasm, and there is also evidence for folate uptake by intact plant cells (23, 24). As for the organisms that cannot create their own folate, especially *Lactobacillus casei* and *L. salivarius*have , the corresponding genome for such activity is mostly unknown (25,26). However, these organisms are almost certainly unrelated to those with mammalian folate carriers, specifically the reduced folate carrier, the folate receptor, the intestinal folate carrier, and the mitochondrial folate carrier (23). The bacteria that cannot make their own folate do not share the same homolog genes as mammals that cannot make folate either (23).

Folate Receptors

Since most bacteria and plant organisms make their own folate, they do not have folate receptors as seen in mammals, especially humans. Throughout the next section, the receptor

proteins discussed will be in reference to the human body. There are currently five known folate receptors on cell membranes, some of which have only been discovered recently (31-35).

Folate receptor alpha, also known as folate-binding protein and folate receptor 1 (Folr1), is a protein encoded by the FOLR gene family that binds folic acid on the surface of the cell and allows it to cross the cell membrane (31). Folate receptor 1 is transcripted from the Folr1 gene (36). This protein receptor has a high affinity for folate, and several reduced folic acid derivatives and allows for the mediated delivery of 5-methyltetrahydrofolate across the cell membrane and into the cell.

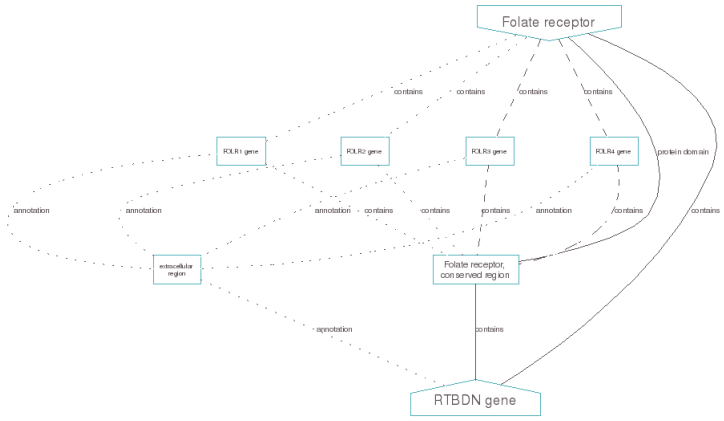

Figure 4 (41). Figure 4 shows various genes and their cooresponding folate receptor for the Folr gene family.

Folate receptor beta, also known as folate receptor 2 (Folr2) is encoded by the Folr2 gene, and is another folate receptor on the surface of most cells (37). Since it is in the same family of genes as Folr1 is, there is very similar functions between Folr1 and Folr2. Folr2 has a 68% and 79% sequence homology with the Folr1 and Folr3 proteins, respectively. The Folr2 protein was originally thought to exist only in placenta, but is also detected in spleen, bone marrow, and thymus (38). This receptor protein is thought to play a role in the transport of methotrexate in synovial macrophages in rheumatoid arthritis patients (38).

Folate receptor gamma is the third member of the Folr gene family. It is also known as folate receptor 3 (Folr3) and is encoded by the Folr3 gene. Having a 71% homology with Folr1, there is many similarities between Folr1, Folr2, and Folr3. The biggest difference between Folr1, and Folr2 and this protein receptor is that this gene includes two polymorphic variants

(33); the shorter one has two base deletion, resulting in a short polypeptide, and a long polypeptide (33). Both protein receptors are secreted in hematopoietic tissues and can help detect certain hematopoietic malignancies (33).

Folate receptor delta, also known as Folr4, is the most recent discovery in the Folr gene family. This protein is produced by the Folr4 gene and has been isolated in various animals, specially the mouse, chicken, lizard, and zebrafish (34). While the gene has been isolated on chromosome 11 in humans (39), the protein receptor has not been isolated on any cells in the human yet. It is, however, predicted to be on human cells.

The final folate receptor isolated so far in the Folr gene family is the Retbindin protein (Rtbdn) (40). The Rtbdn gene is located on chromosome 19. While this gene was first identified in a study of human eye tissues, the protein has been found to be expressed all over the body, especially in the nervous and secretory cells and retina. (35). Along with transporting folate, this gene is predicted to play a role in binding retinoids, as it shares some homology with riboflavin binding proteins (35).

There is at least one known reduced folate carrier protein, SLC19A1, which is also known as reduced folate carrier (RFC), FOLT, and Intestinal folate carrier (41). This protein binds to folate and through the carrier-mediated method, transports it across the cell membrane through folate receptors, regulating how much folate a cell gets (42). Located on chromosome 21, this gene that encodes for SLC19A1 has three transcript variants which all for three different isoforms to be transcribed (43). SLC19A1 is highly expressed in the intestines, where it binds to the folate that is taken up for the cells to use. In a study where the SLC19A1 gene was knocked out, mice had a significantly less uptake of folate (up to 90%), which shows that this carrier protein is necessary for folate uptake into the cells (43). SLC19A1 is not just expressed

in the intestines; it has been isolated in the placenta and in many major organs of developing fetuses (44). This is probably due to folate being required for cell division and growth, and in early development, cells are rapidly dividing in fetuses.

Below is a chart, figure 5, showing various cells in the human body and how much folic acid receptor proteins are expressed (31-36). The units for expression values relate to fluorescence intensity. In the microarray, there are probes that measure the intensity for the receptor they are studying. These intensity values are summarized using various data processing algorithms. In these studies, the Affymetrix array was used, and its units are gcrma. The higher the number, the more the protein is expressed (45). Gcrma is on a scale from 0 to 1000. The colors denote the type of cell.

	Folr1	Folr2	Folr3	RTBDN	SLC19A1 Carrier Protein
Bone Marrow	95	65	64	48	14
Whole Blood	280	67	442	41	72
Lymph Node	9	102	17	49	8
Thymus	269	99	15	66	8
Brain	332	39	16	43	8
Cerebellum	191	47	15	69	8
Retina	267	45	22	361	14
Spinal Cord	13	67	22	45	10
Heart	511	227	29	41	16
Smooth Muscle	147	26	22	42	11
Skeletal Muscle	76	61	40	51	11
Small Intestine	290	40	18	38	14
Colon	274	22	18	36	10
Adipocyte	129	48	22	44	11
Kidney	345	55	20	39	8
Liver	415	58	28	42	63
Lung	279	92	22	57	36

Pancreas	134	45	16	54	10
Thyroid	87	33	23	51	13
Salivary Gland	36	53	15	53	8
Adrenal Gland	184	89	16	44	8
Skin	69	45	17	45	8
Ovary	21	47	13	56	8
Uterus	175	27	15	44	7
Placenta	161	278	19	50	23
Prostate	316	28	22	34	13
Testis	129	43	16	79	11
Tonsil	49	60	22	43	10
Myeloid	266	43	55	32	55
Monocytes	184	35	718	29	41
Dentritic Cells	347	22	20	33	10
NK Cells	338	9	9	33	11
T Cells (CD4+)	184	18	18	26	9
T Cells (CD8+)	150	17	17	32	8
B Lymphoblasts	144	19	19	34	27
B Cells	226	29	20	34	9
Endothelial	255	63	19	35	36
Fetal Brain	10	37	19	49	9
Prefrontal Cortex	59	33	19	40	10
Cingulate Cortex	50	103	20	57	10
Parietal Lobe	29	46	21	42	11
Temporal Lobe	64	69	17	47	9
Occipital Lobe	33	56	17	40	9
Ciliary Ganglion	11	44	16	43	7
Cerebellum Peduncles	33	100	22	43	13
Globus Pallidus	30	28	17	39	7
Olfactory Bulb	33	50	15	43	7
Thalamus	16	96	19	37	9
Hypothalamus	10	48	21	43	10
Subthalamic Nucleus	45	78	17	43	8
Caudate Nucleus	10	83	17	42	9
Amygdala	47	52	18	69	10
Pons	73	87	18	43	11
Medulla Oblongata	113	64	18	53	9
Sup Cervical Ganglion	16	60	22	61	12
Dorsal Root Ganglion	14	57	19	56	8
Trigeminal Ganglion	31	46	24	42	11
Cardiac Myocytes	69	49	24	33	16

Atrioventricular Node	12	71	17	44	10
Tongue	147	63	20	41	13
Fetal Liver	253	123	16	61	9
Fetal Lung	56	76	17	49	9
Trachea	327	63	18	54	8
Bronchial Epithelium	252	35	66	39	9
Appendix	16	61	19	44	13
Fetal Thyroid	83	33	19	41	12
Pancreatic Islet	67	51	21	89	11
Adrenal Cortex	91	59	25	38	11
Pineal (Day)	44	33	22	2306	11
Pineal (Night)	93	46	22	1583	10
Pituitary	121	78	22	63	11
Uterus Corpus	43	55	17	48	11
Testis Seminif Tubule	21	47	17	43	10
Testis Germ	82	34	15	43	9
Testis Intersitial	87	33	16	48	8
Testis Leydig	20	72	21	41	14

Figure 5 (31-36, 41). Folr4 expression is not in this chart as information to its expression is not yet available.

Folr1 expression is very high, especially in the blood, nervous system, and muscle cells. It is expressed the highest in the heart, followed by immune system cells. Folr2 has high expression rates for fetal cells, and well as the nervous system. Monocytes express Folr3 receptor at an exceptionally high rate.

Folic Acid Receptor in Macrophages

Macrophages are differentiated monocytes that engulf and destroy dead cells, other cellular debris, and pathogens (46) through digesting the particles. Monocytes express in large numbers folic acid receptors, especially folr1 and folr3. One reason for this large expression of folic acid is for the high rate of cell division and specialization of monocytes (47). Macrophages also express folic acid receptors in high quantities, as folic acid is a requirement for cell life. It is known that macrophages target debris and pathogens based off of size and shape (48).

However, there does not seem to be any known link between macrophage's bacteria detection and folic acid receptors. Because the bacteria cells have cell walls that folic acid cannot cross between, it seems unlikely that macrophages could detect the folic acid production of bacteria with the cell wall in the way.

Conclusion

While there are five known folic acid receptors (and potentially more to be discovered) it is unknown as to why the body has multiple receptors that do the same thing. Why do monocytes express so many Folr3 receptors and other cells like the heart express so many Folr1? In regards to the bacteria that lost the ability to create their own folic acid, how do they take in folic acid and what receptors do they use? These questions can lead the way to further research on folate and uncover new knowledge on a chemical required by all life.

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